余電流もAに比較して十分に小さい値となっている。そして、これらの傾向は高純度の黒鉛を用いたC，Dにおいて特に顕著である。

第3図は前記A～Dの酵素電極について、電位設定30分後の残余電流が定常値となった時点でグルコースを添加し、その濃度を2×10^{-4}モル/lとしたときの応答特性を示す。酵素が存在しないときには、図に示すような電流増加は認められない。図から明らかなごとく、A～Dいずれの電極においても電流増加にほとんど差はないが、応答電流（電流増加）と残余電流の大きさを比較すると、AとB，C，Dとの差は明らかである。C，Dにおいては、残余電流が特に小さいため、さらに低濃度の基質をも感度よく検出することができる。

以上のごとく、電子伝導性物質として用いるカーボンにより、酵素電極の性能が左右されるが、黒鉛の使用により電極性能を大幅に向上することができ、中でも固定炭素99.5重量％以上、灰分0.005重量％以下の高純度黒鉛を用いた場合の効果が大である。

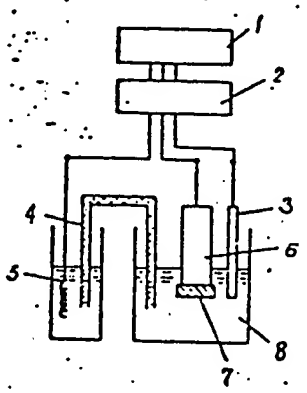
本発明は、実施例で説明したグルコースオキシダーゼについて限定されることはなく、酸化還元酵素とレドックス化合物を共存させ、その酸化還元電流を検出するいかなる酵素電極系においても適用することができる。また、アルコール脱水素酵素などのように補酵素を必要とする酵素系の場合には、ニコチンアミドアデニンジヌクレオチド（NAD）などの酵素に対応する補酵素をさらに加えて固定化すればよい。レドックス化合物としてはクロルアニルの他ブロムアニル、あるいは各種レドックスポリマーなどの不溶性レドックス化合物を用いることにより、繰り返し使用の可能な、酵素電極とすることができる。

4．図面の簡単な説明

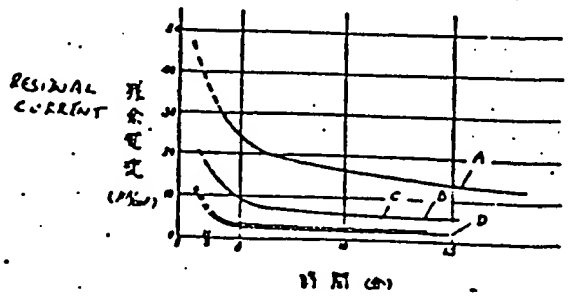
第1図は基質濃度の測定系の構成を示す図、第2図は各種酵素電極についての電位設定後の残余電流の変化を比較した図、第3図は酵素電極のグルコースに対する応答特性を比較した図である。

代理人の氏名　弁理士　中　尾　敏　男　ほか1名

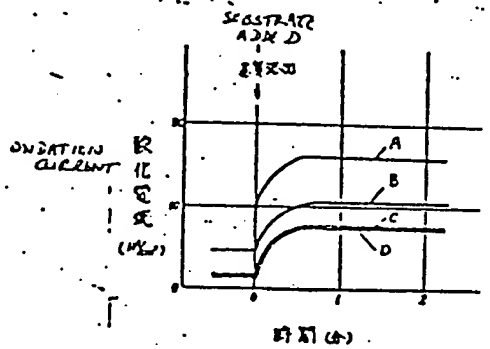
第1図



第2図



第3図



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Specification

1. Name of Invention

Enzyme electrode

2. Scope of Claim

1) An enzyme electrode, characterised in that it possesses a fixed oxidation-reduction enzyme, a redox compound used with this, and an electron conductive material, where this electron conductive material is graphite.

2) An enzyme electrode as in 1) of the claim where the co-enzyme of the oxidation-reduction is fixed.

3) An enzyme electrode as in 1) and 2) of the claim where the graphite is a high purity graphite of fixed carbon above 99.5% by weight and ash content of below 0.005% by weight.

4) An enzyme electrode as in 1) and 2) of the claim where the redox compound is an insoluble redox compound.

3. Detailed Specification

This invention is intended to provide an enzyme electrode which is electrochemically active against the substrate affected by the specific catalytic action of the enzyme, and which will enable rapid and simple measurement of the substrate concentration, and which will serve for continuous and repeated use. This invention also relates to an enzyme electrode which can be used in a cell, which will convert the chemical energy of a substrate into electrical energy by virtue of the combination with the enzyme electrode, etc.

Recently, with the advances in applications technology of various enzymes, trials have been carried out on industrial uses of the specific catalytic action possessed by these enzymes. For example, attempts have been made in the detection of substrate concentration where this substrate is a substance which reacts specifically with the enzyme.

In order to treat an enzyme reaction as an electrochemical reaction, a

suitable redox compound, for example, may be introduced into the enzyme reaction system, and the redox reaction of this redox compound may be detected electrochemically. An example of this is the case where the substrate is glucose, the redox compound is benzoquinone, and the enzyme is glucose oxidase, where the reactions expressed by equations (1) and (2) below are made to occur in a mixture between the electrodes:

(1) glucose + benzoquinone $\xrightarrow{\text{glucose oxidase}}$ gluconolactone + hydroquinone

(2) hydroquinone $\dashrightarrow$ benzoquinone + $2H^+$ + 2e

The hydroquinone produced as a reduction product in equation (1) is oxidised electrochemically into benzoquinone in equation (2), and the glucose concentration can be detected at this time as the oxidation current.

However, what is desirable in practice is to fix the expensive enzymes and redox compounds together to produce an enzyme electrode which will be easy to handle and capable of repeated use. In order to fix together the enzyme and the redox compound, a suitable electron conductive material is necessary as a collector. Carbon, unlike metals, dissolves during anodic oxidation and does not form any inert film; it has stable characteristics as an electrode material and is a most favourable electron conductor.

Enzyme electrodes using carbon may be constructed, for example, by the method of press-moulding a mixture of carbon powder and the redox compound and fixing the enzyme on this moulding; or by the method where carbon powder, on to which the enzyme has already been fixed, is mixed into the mixture as above, and the whole is subsequently press-moulded.

The method of measurement consists in the above electrode being kept in a fixed location with respect to the saturated calomel electrode in the buffer solution, the concentration of the substrate being varied, and the consequent variations in the anode current being measured.

The following points are raised as questions to be considered in this type of measurement: first there is the matter of the interval between the electrode installation in its fixed position and the achievement of conditions when measurement may be made; and secondly there is the matter of the size of the residual current when the substrate of interest is not included in the detection solution. These are both important points in the use of enzyme electrodes as

they determine the convenience, the rapidity, or the sensitivity and S/N ratio.

Either acetylene black or graphite powder might be used as the electron conductive substance, which is one of the structural materials of this enzyme electrode. However, these carbons contain numerous impurities, mainly heavy metals; in particular, the surface area of acetylene black is large, and active functional groups are present in the surface. Where such carbons are used as electron conducting materials, because currents flow originating in the oxidation and reduction of the active functional groups and in the impurities in the carbon, considerable obstacles occur in the way of measurements. Moreover, because heavy metal ions act as blocking agents on many enzymic reactions, it is not desirable that heavy metals be included in the carbon as impurities.

This invention increases the performance of the enzyme electrode by using graphite as the electron conducting material. In particular, by the use of high purity graphite where the fixed carbon is over 99.5% by weight and the ash content is below 0.005% by weight, the residual current can be very much diminished and the (electrodes') efficiency can be greatly increased.

Below, this invention is described using a practical embodiment.

The enzyme electrode was prepared by the method to be related below. Carbon powder was well mixed in the proportion of 4 : 1 by weight with chloranil as the insoluble redox compound and press-moulded. Then glucose oxidase as the enzyme was fixed on to the moulding obtained by means of glutaraldehyde. The enzyme electrode thus obtained was fitted to an electrode support and used for carrying out measurements in the measurement system shown in Fig. 1. In Fig. 1, 1 is the recorder, 2 is the potentiostat, 3 is the reference electrode, 4 is the salt bridge, 5 is the opposite electrode, 6 is the electrode support fitted to the enzyme electrode 7, and 8 is the phosphoric buffer solution of pH 5.6 which contains the glucose substrate.

Fig. 2 shows the relationships with time of the residual currents of the enzyme electrodes prepared with various types of carbon powder as the electron conducting material, where the potential was set at 0.40V in a solution which did not contain the substrate.

In the figure, A is the case where the electron conducting material is

acetylene black, B is artificial graphite of fixed carbon above 99.0% by weight ash content below 0.2% by weight, C is high purity graphite of fixed carbon above 99.5% by weight and ash content below 0.005% by weight, D is high purity graphite of fixed carbon above 99.5% by weight and ash content below 0.001% by weight.

As is clear from the figure, in the case of A, the residual current takes a long time to decline and reach a constant value, whereas with B, C, and D, an approximately constant value was reached in 5~8 minutes after the voltage setting; and the residual currents in these cases were sufficiently smaller than that of case A. These trends were particularly clear in the cases of C and D where high purity graphite was used.

Fig. 3 shows the reaction characteristics when, 30 minutes after the voltage setting, when the residual currents had attained constant values, glucose was added up to a concentration of 2×10^{-4} mol/litre. When the enzyme is not present, no current increase such as that shown in the figure is seen. As is clear from this figure, there is almost no difference between the current increases in the cases of electrodes A~D, but when the size of the response currents (current increases) and residual currents are compared, differences are apparent between A, B, C, and D. In C and D, as the residual current is particularly small, smaller concentrations of the substrate are detectable with good sensitivity.

As related above, the performance of an enzyme electrode is governed by the carbon used as the electron conducting material, and it is possible to increase greatly the electrode performance by the use of graphite; and of these, the greatest effect was in the case of the use of a high purity graphite of fixed carbon above 99.5% by weight, and ash content below 0.005% by weight.

This invention is not limited to the glucose oxidase used in this explanatory embodiment, but it is suited to any enzyme electrode system where a redox enzyme is placed with a redox compound, and which detects the oxidation-reduction current. Moreover, in the case of an enzyme system where a co-enzyme is necessary, such as with alcohol dehydrogenase, etc. the co-enzyme corresponding to nicotineamide adenine dinucleotide (NAD) etc., may also be added and fixed. By the use of insoluble redox compounds apart from chloranil, such as bromanil or redox polymers as the redox compounds, it is possible to obtain an enzyme electrode capable of repeated use.

4. Simple Explanation of Figures

Fig. 1 shows the structure of a system for measurement of substrate concentration; Fig. 2 shows the variations in residual current after voltage setting for various types of enzyme electrode; and Fig. 3 shows a comparison of the response characteristics of the enzyme electrodes against glucose.

Name of Agent: Nakao Toshio, Attorney-at-Law, and one other.